Electronic Supplementary Information (ESI)

# Comparative Understanding of Peroxide Quantitation Assays: A Case Study with Peptide Drug Product Degradation

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Table S1. Example assay plate design for validation studies in water.

|	1	2	3	4	5	6	7	8	9	10	11	12
A	Ctrl 1.5 ppm			N/A			0.0078			0.0156		
B	0.031			0.0625			0.125			0.25		
C	0.5			1			2			Ref 2		
D	Ref 1			Ref 0.5			Ref 0.25			Ref 0.125		
E	Ref 0.0625			Ref 0.031			Ref 0.0156			Ref 0.0078		
F	Precision 0.031			Precision 0.031			Precision 0.031			Precision 0.031		
G	Precision 0.031			N/A			Stability 0.05			Stability 0.05		
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
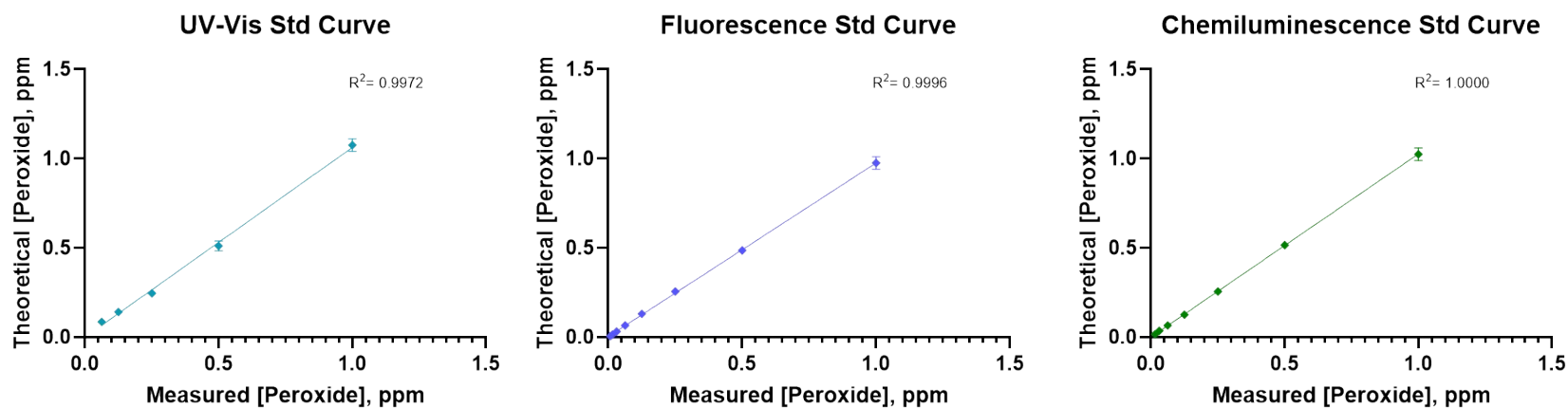
Figure S1. Standard curves for linearity assessments during validation studies of UV-Vis, fluorescence and chemiluminescence assays for peroxide quantification.

Table S2. Forced degradation data (presented in Figure 2) for peptide drug products in presence of peroxide under different degradation conditions, 5 °C-21 days, 40 °C-7 days, and 40 °C-21 days.
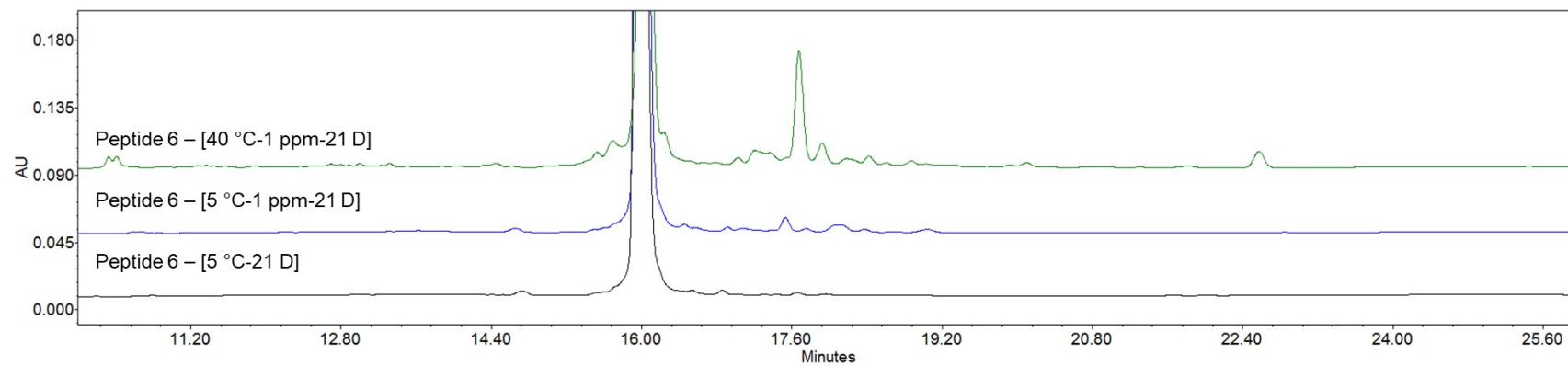
Peptide DPs 21 D	5 °C	5 °C – 1 ppm	5 °C – 10 ppm	Peptide DPs 7 D	40 °C	40 °C – 1 ppm	40 °C – 10 ppm	Peptide DPs 21 D	40 °C	40 °C – 1 ppm	40 °C – 10 ppm
Insulin	96.4	96.4	96.4	Insulin	95.6	95.2	92.6	Insulin	92.1	92.0	84.9
Peptide 1	96.9	96.3	96.4	Peptide 1	96.8	96.1	95.9	Peptide 1	92.2	91.2	87.8
Peptide 2	95.8	95.7	94.9	Peptide 2	94.8	94.8	91.2	Peptide 2	85.6	84.3	73.1
Peptide 3	92.9	92.3	90.2	Peptide 3	92.7	91.9	91.0	Peptide 3	88.3	85.8	80.9
GLP-1(7-36)	80.0	80.1	79.5	GLP-1(7-36)	80.0	78.2	78.4	GLP-1(7-36)	77.5	74.9	73.1
Peptide 4	89.8	89.6	88.2	Peptide 4	88.7	83.5	71.6	Peptide 4	83.9	77.3	55.4
Peptide 5	98.9	98.1	98.1	Peptide 5	89.8	80.2	41.6	Peptide 5	89.9	50.7	17.1
Peptide 6	92.0	86.7	67.5	Peptide 6	86.9	77.3	29.6	Peptide 6	87.4	64.2	19.1
Peptide 7	92.3	85.6	45.1	Peptide 7	70.8	55.7	6.4	Peptide 7	41.2	27.9	0.2
Peptide 8	87.1	81.7	46.6	Peptide 8	38.8	30.3	3.0	Peptide 8	51.9	34.3	0.1
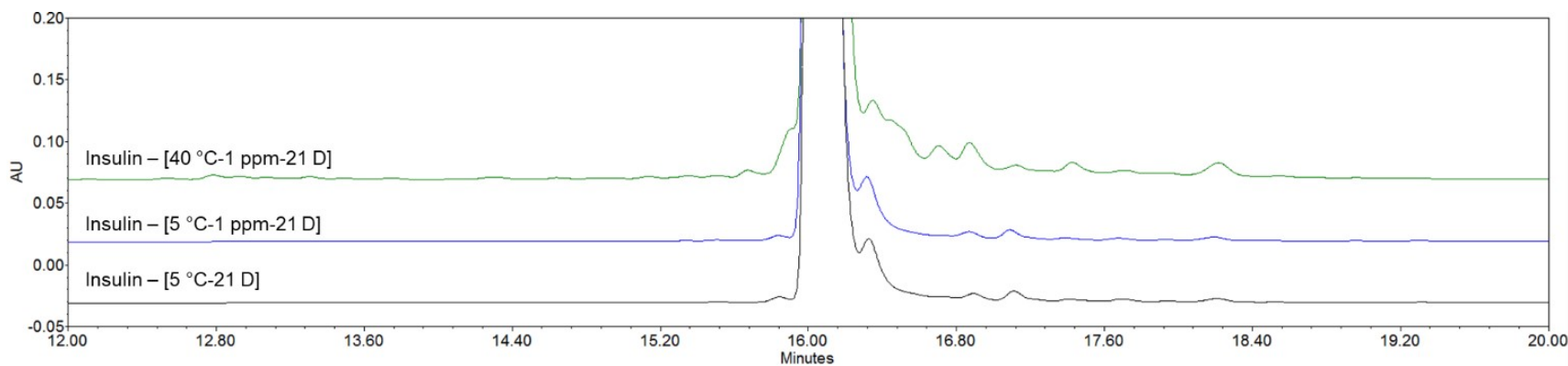
Figure S2. RP-HPLC chromatograms showing low degradation for Insulin in presence of 1 ppm peroxide.
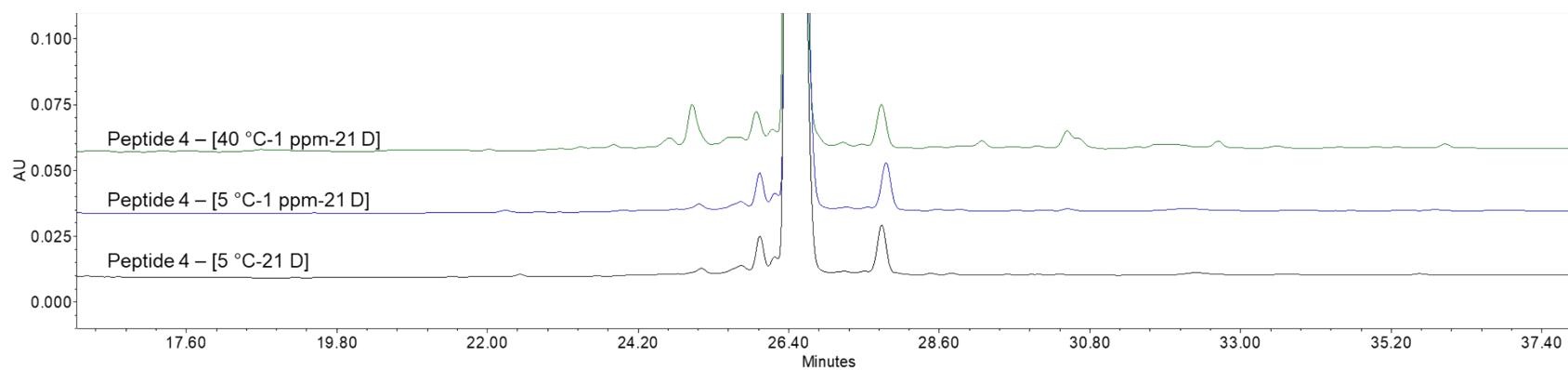
Figure S3. RP-HPLC chromatograms showing moderate degradation for Peptide 4 in presence of 1 ppm peroxide.

Figure S4. RP-HPLC chromatograms showing high degradation for Peptide 6 in presence of 1 ppm peroxide.

Table S3. Peroxide quantitation data for peptide drug products under different degradation conditions, 5 °C-21 days, 40 °C-7 days, and 40 °C-21 days: top three plots are for Fe-XO (UV-Vis) assay, and bottom three are for HyPerBlu (luminescence) assay, respectively (ND=not detected).

Peptide DPs 21 D (UV-Vis)	5 °C	5 °C – 1 ppm	5 °C – 10 ppm
Insulin	0.08	0.80	8.01
Peptide 1	0.12	0.74	8.06
Peptide 2	0.10	0.81	8.07
Peptide 3	0.12	0.96	7.97
GLP-1(7-36)	0.1	0.93	8.4
Peptide 4	0.11	0.94	7.98
Peptide 5	0.11	0.95	8.19
Peptide 6	0.08	0.71	7.08
Peptide 7	0.09	0.67	6.63
Peptide 8	0.08	0.71	6.93

Peptide DPs 7 D (UV-Vis)	40 °C	40 °C – 1 ppm	40 °C – 10 ppm
Insulin	0.08	0.78	4.66
Peptide 1	0.12	0.83	4.69
Peptide 2	0.11	0.90	4.72
Peptide 3	0.12	0.90	4.77
GLP-1(7-36)	0.09	0.83	4.68
Peptide 4	0.11	0.72	4.78
Peptide 5	0.09	0.83	4.83
Peptide 6	0.09	0.31	4.59
Peptide 7	0.08	0.34	4.60
Peptide 8	0.08	0.36	4.66

Peptide DPs 21 D (UV-Vis)	40 °C	40 °C – 1 ppm	40 °C – 10 ppm
Insulin	0.08	1.20	12.59
Peptide 1	0.14	1.28	13.07
Peptide 2	0.16	1.37	12.56
Peptide 3	0.15	1.33	11.56
GLP-1(7-36)	0.14	1.08	10.78
Peptide 4	0.15	0.63	6.38
Peptide 5	0.08	1.30	13.12
Peptide 6	0.08	0.21	5.28
Peptide 7	0.08	0.12	8.62
Peptide 8	0.08	0.13	8.88

Peptide DPs 21 D (Lumi)	5 °C	5 °C – 1 ppm	5 °C – 10 ppm
Insulin	ND	0.73	3.15
Peptide 1	0.06	1.02	3.95
Peptide 2	0.02	1.13	3.01
Peptide 3	ND	0.95	3.06
GLP-1(7-36)	ND	0.74	2.65
Peptide 4	0.09	3.32	3.91
Peptide 5	0.02	1.22	3.64
Peptide 6	ND	0.69	2.69
Peptide 7	ND	0.70	2.23
Peptide 8	ND	0.62	2.33

Peptide DPs 7 D (Lumi)	40 °C	40 °C – 1 ppm	40 °C – 10 ppm
Insulin	ND	0.74	3.34
Peptide 1	0.07	1.15	4.42
Peptide 2	0.05	1.10	3.59
Peptide 3	0.01	0.88	3.23
GLP-1(7-36)	0.03	0.67	2.91
Peptide 4	0.07	2.29	3.00
Peptide 5	ND	0.39	3.02
Peptide 6	ND	0.28	2.01
Peptide 7	ND	0.36	2.30
Peptide 8	ND	0.33	2.25

Peptide DPs 21 D (Lumi)	40 °C	40 °C – 1 ppm	40 °C – 10 ppm
Insulin	0.01	1.21	5.85
Peptide 1	0.06	1.68	6.64
Peptide 2	0.14	1.68	5.49
Peptide 3	0.02	1.33	5.09
GLP-1(7-36)	0.05	1.04	4.75
Peptide 4	0.08	2.18	3.60
Peptide 5	ND	0.60	4.26
Peptide 6	0.01	0.20	2.27
Peptide 7	ND	0.08	3.76
Peptide 8	ND	0.09	3.87